- 1 Supplementary Materials: Integrated multi-omic analyses of bovine
- 2 milk identify biomarkers of negative energy balance.
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- 11 Supplementary Figure 1. Scree plot of redundancy analysis (RDA) component versus percentage of
- 12 variance explained for the long and moderate (M) feed restriction trial.



15 Supplementary Figure 2. Scree plot of redundancy analysis (RDA) component versus percentage of

 $\,$ variance explained for the short and intense (H) feed restriction trial.



Supplementary Figure 3. Circle correlation plots of the top 25 features selected for pairwise sparse partial least squares (sPLS) analyses of each pair of data blocks for the long and moderate (M) trial. The block labeled "other" corresponds to milk yield, contents, major protein, and metabolite data.



Supplementary Figure 4. Tuning of number of DIABLO components for the long and moderate (M) trial based on leave-one-out cross validation and evaluation criteria including the overall balanced error rate (BER) and error rate (ER) based on different classification metrics based on (1) the largest predicted dummy variable (max.dist), (2) the minimal Euclidean distance between class centroids and predictions (centroids.dist), and (3) the minimal Mahalanobis distance between class centroids and predictions (mahalanobis.dist). Here, the optimal number of components is identified by all metrics as being 3.



30 Supplementary Figure 5. Visualization of the correlation between the first DIABLO component for each 31 data block in the long and moderate (M) trial, with samples colored according to period (blue, before 32 feed restriction; orange, during feed restriction). Values along the lower triangular value correspond 33 to Pearson's correlation coefficients. The block labeled "other" corresponds to milk yield, contents, 34 major protein, and metabolite data.



36 Supplementary Figure 6. Projection of samples into the space spanned by the first and second DIABLO 37 components of each data block in the long and moderate (M) trial, with samples colored according to 38 period (blue, before feed restriction; orange, during feed restriction). The block labeled "other" 39 corresponds to milk yield, contents, major protein, and metabolite data.



41 Supplementary Figure 7. Projection of samples into the space spanned by the first and third DIABLO 42 components of each data block in the long and moderate (M) trial, with samples colored according to 43 period (blue, before feed restriction; orange, during feed restriction). The block labeled "other" 44 corresponds to milk yield, contents, major protein, and metabolite data.

Correlation Circle Plot



Supplementary Figure 8. Correlation circle plot of selected features from each block for the first and
second DIABLO components in the long and moderate (M) trial, with features colored according to
data block (blue, FG miRNome; orange, whole milk miRNome; grey, skim milk proteome; green, milk
yield/contents and metabolite concentrations).



52 Supplementary Figure 9. Circle correlation plots of the top 25 features selected for pairwise sparse 53 partial least squares (sPLS) analyses of each pair of data blocks for the short and intense (H) trial. The 54 block labeled "other" corresponds to milk yield, contents, major protein, and metabolite data.







Supplementary Figure 11. Visualization of the correlation between the first DIABLO component for each data block in the short and intense (H) trial, with samples colored according to period (blue, before feed restriction; orange, during feed restriction). Values along the lower triangular value correspond to Pearson's correlation coefficients. The block labeled "other" corresponds to milk yield, contents, major protein, and metabolite data.



Supplementary Figure 12. Projection of samples into the space spanned by the first and second DIABLO components of each data block in the short and intense (H) trial, with samples colored according to period (blue, before feed restriction; orange, during feed restriction). The block labeled "other" corresponds to milk yield, contents, major protein, and metabolite data.



76 Supplementary Figure 13. Projection of samples into the space spanned by the first and third DIABLO components of each data block in the short and intense (H) trial, with samples colored according to 77 period (blue, before feed restriction; orange, during feed restriction). The block labeled "other" 78 79 corresponds milk metabolite to yield, contents, data. major protein, and

Correlation Circle Plot



80 81 Supplementary Figure 14. Correlation circle plot of selected features from each block for the first and second DIABLO components in the short and intense (H) trial, with features colored according to data 82 83 block (blue, FG miRNome; orange, whole milk miRNome; grey, skim milk proteome; green, milk yield/contents and metabolite concentrations). 84



Supplementary Figure 15. Projection of samples into the space spanned by the first and second MINT components across data blocks in both the long and moderate (M, triangles) and short and intense (H, circles) trials. Samples are colored according to period (orange, before feed restriction; blue, during feed restriction), with different symbols representing each trial.